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**ON THE DEVELOPMENT OF PARASITIC COPEPODA II.
COLOBOMATUS PUPA IZAWA (CYCLOPOIDA:
PHILICHTHYIDAE)^{1,2)}**

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With Text-figures 1-27

Following the previous and first paper of the present serial studies, dealing with the free-living stages of *Sarcotaces*, this paper describes the free stages of a species of *Colobomatus* which belongs, together with *Sarcotaces*, to the family Philichthyidae of the Copepoda.

Colobomatus Hesse, 1873, is an endoparasitic genus, found in the cephalic sensory canal system of fishes. Because of such a secret habitat, in addition to smallness of the animal body, it is difficult to detect specimens of this genus only by surface examination of host fishes, and thus little has been known in general about the genus. Though twenty-four species have been described so far, no larvae have ever been found or described. In 1971 three species of this genus were found and described as new species for the first time from the Japanese waters (Izawa, 1974). One of which, *C. pupa* Izawa obtained from the Japanese goat fish, *Pseudupeneus spilurus* (Bleeker), was examined throughout its whole free-living developmental stages traced by rearing larvae.

Material and Methods

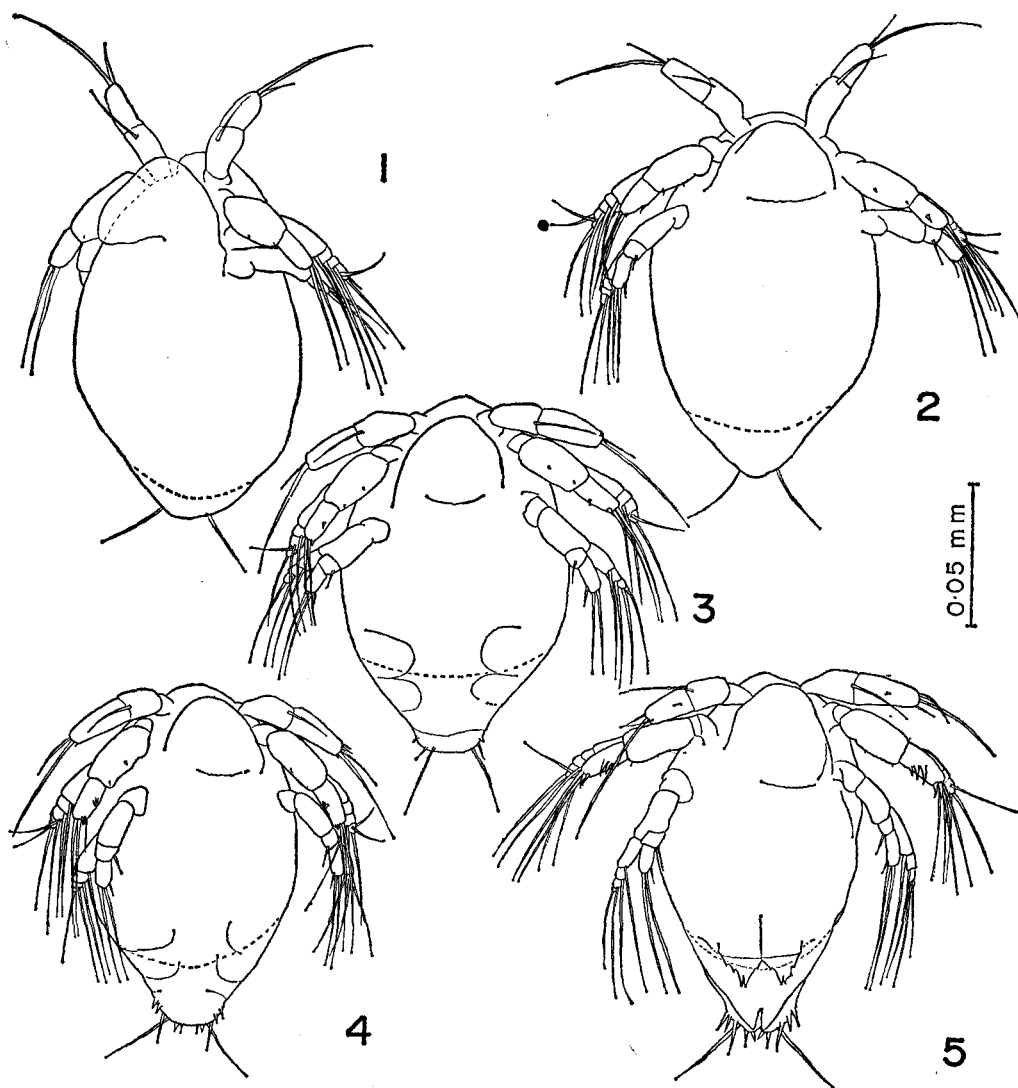
The larvae for the following descriptions were reared at the Seto Marine Biological Laboratory in November, 1971. Twenty-one specimens including ovigerous females of *Colobomatus pupa* were obtained from the cephalic sensory canal system of *Pseudupeneus spilurus* collected in Tanabe Bay, Wakayama prefecture. They were washed by using a pipette into a glass dish under stereomicroscope from the canal system which was removed from the host body together with its pre- and suborbital bones and preopercles and then cut open carefully. The eggs obtained from females were reared to the first copepodid stage in a glass bowl with sea water filtered through cotton. The bowl was kept in water bath at 16-18°C. Neither aeration nor circu-

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lation of the water was made, but the water was renewed two or three times a day. As the eggs contain a considerable amount of yolk, the larvae are lecithotrophic and can be brought up to the copepodid stage without any food supply.

The larvae of respective developmental stages were fixed with formalin and preserved in alcohol and later they were examined by being immersed in lactic acid on the wooden slide of Humes & Gooding, in some cases being stained with Chlorazol Black E, all nearly the same as in the case of *Sarcotaces*. The castoff covering at ecdyses was observed for detailed morphology, especially of appendages.



Figs. 1-5. Nauplius stages of *Colobomatus pupa* Izawa. 1. First nauplius, ventral view. 2. Second nauplius, ventral view. 3. Third nauplius, ventral view. 4. Fourth nauplius, ventral view. 5. Fifth nauplius, ventral view. The scale applicable to all figures.

Development

In the present material, the number of eggs carried by single females was relatively small, about fifty at the maximum. The eggs packed loosely in the egg sac of very thin membrane are ovoid, 0.12 mm long and 0.08 mm in diameter, and colored yellowish as they contain a considerable amount of yolk.

Newly hatched nauplii were able to swim and reached to the first copepodid stage within five days through five nauplius stages, but no growth of the body was seen during the development. The first copepodids were capable of active swimming and thus probably they might be the infective stage. No pigments appeared throughout these stages. The first nauplius survived all these stages to the first copepodid at a higher rate.

Description of Developmental Stages

First Nauplius Stage (Figs. 1, 6, 11, 16) %

Length 0.13 mm and width 0.08 mm on an average of 5 measurements. Plump body is ovoid and provided with three pairs of appendages as in typical nauplii. Labrum is not prominent. Furcal armature consists of a single pair of setae.

First antenna (Fig. 6) consists of a short simple basal segment, not yet separated from body, and two longer distal segments covered with short rows of fine bristles. Second segment almost equal to third segment and with a seta at the distal end of the ventral margin. Terminal segment with two apical plumose setae, of which the longer one is furnished with a short aesthete near the base.

Second antenna (Fig. 11) biramous. Protopodite 2-segmented. Coxopodite short, with short rows of fine bristles on the surface. Basipodite with two minute spinules at the middle and the distal corner of the inner margin. Endopodite 1-segmented, with a setule at the middle on the inner margin and two long apical setae plumosed. Exopodite 5-segmented; first segment as long as endopodite, but without distinct joint to basipodite, four distal segments short. Each of five segments with a long plumose seta at the inner distal corner, only terminal segment with an additional short seta on the outer distal corner.

Mandible (Fig. 16) biramous. Protopodite 2-segmented; coxopodite short, basipodite longer; both unarmed. Endopodite 2-segmented. First segment articulated obliquely and subterminally to basipodite, about one third as long as, but wider than second segment, and with a seta at the inner distal corner. Second segment with a short seta near the inner distal corner and two long apical setae plumosed. Exopodite almost as long as basipodite and 4-segmented, segments diminishing the size distally and each with a long plumose seta at the inner distal margin in proximal three but at the distal end in terminal segment.

Second Nauplius Stage (Figs. 2, 7, 12, 17)

Length 0.13 mm and width 0.08 mm on an average of 5 measurements. Body is also ovoid but more narrowed caudally. Labrum and furcal armature as in first

stage.

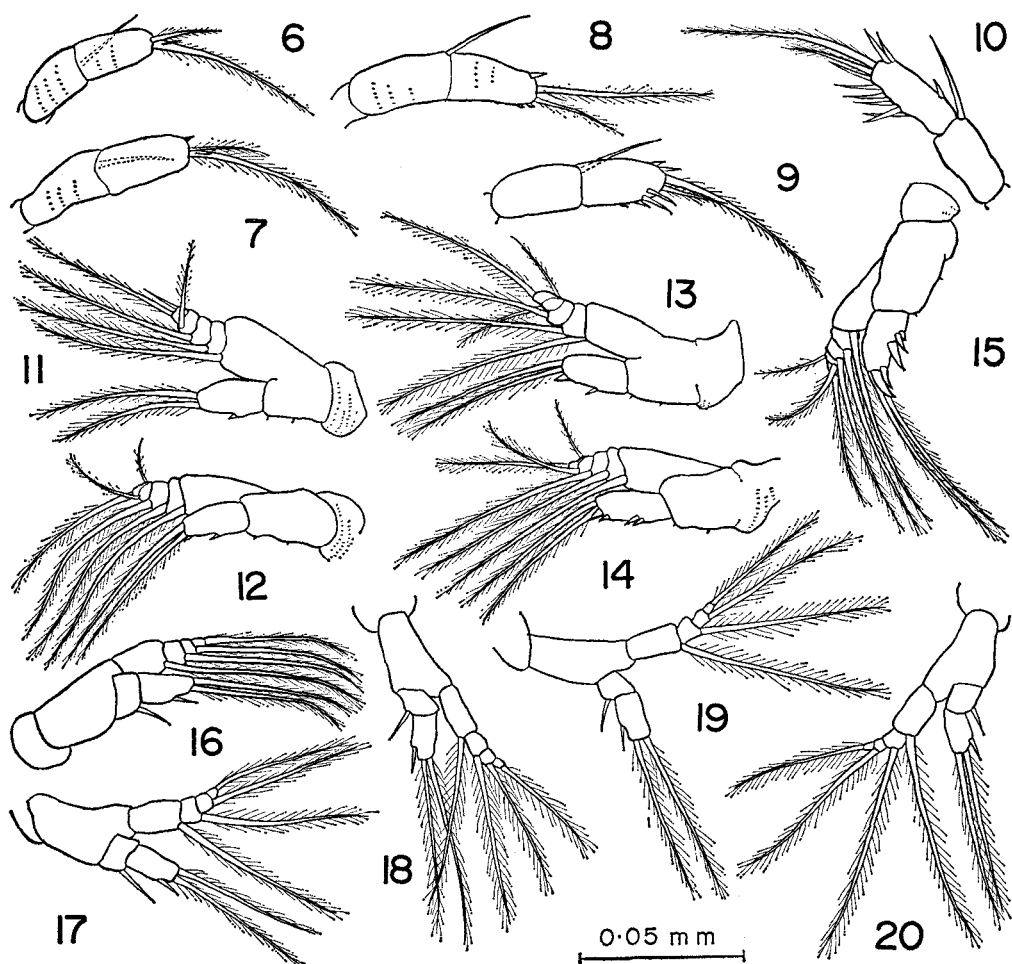
First antenna (Fig. 7) with an additional minute setule at the ventro-distal corner.

Second antenna (Fig. 12) with an additional minute setule at the inner distal corner of endopodite. A short terminal seta of exopodite in first nauplius changed to a short plumose seta issued from the outer proximal side of terminal segment.

Mandible (Fig. 17) as in first mauplius.

Third Nauplius Stage (Figs. 3, 8, 13, 18)

Length 0.13 mm and width 0.08 mm on an average of 5 measurements. Caudal margin more or less truncate and with four additional setules in furcal armature.



Figs. 6-20. Nauplius stages of *Colobomatus pupa* Izawa. 6-10. First antennae of 1st-5th nauplii; 6, 7—dorsal view, 8, 10—anterior view, 9—ventral view. 11-15. Second antennae of 1st-5th nauplii; 11, 13—anterior view, 12, 15—ventral view. 16-20. Mandibles of 1st-5th nauplii, anterior view. The scale applicable to all figures.

First antenna (Fig. 8) with two additional setules on the dorso-distal margin.

Second antenna (Fig. 13) changed slightly; a setule at the inner distal corner of endopodite rather stout.

Mandible (Fig. 18) as in the preceding stage.

Fourth Nauplius Stage (Figs. 4, 9, 14, 19)

Length 0.13 mm and width 0.07 mm on an average of 5 measurements. Body slightly narrower, and furcal armature gained six more setules.

First antenna (Fig. 9) with three additional setules on terminal segment, one at the ventro-distal corner and two near the middle on the dorsal margin; two setules appeared in the preceding stage at the dorso-distal corner much enlarged.

Second antenna (Fig. 14) with two additional setules on exopodite, respectively at the middle and near the inner distal corner.

Mandible (Fig. 19) unaltered.

Fifth Nauplius Stage (Figs. 5, 10, 15, 20)

Length 0.13 mm and width 0.07 mm on an average of 5 measurements. Caudal margin concaved at the middle and with more developed setules in furcal armature. Two rudimentary legs emerged on the postero-ventral surface. Labrum as in the preceding stages. Mouth opening and alimentary canal still undefinable. No signs of post-mandibular oral appendages recognized.

First antenna (Fig. 10) with three additional setules on terminal segment, short one on the ventro-proximal margin and two longer ones in the row of setules in the distal half of the dorsal margin.

Second antenna (Fig. 15) with an additional setule proximal to the two stouter transmitted ones near the middle on the inner margin of endopodite.

Mandible (Fig. 20) unaltered.

First Copepodid Stage (Figs. 21–27)

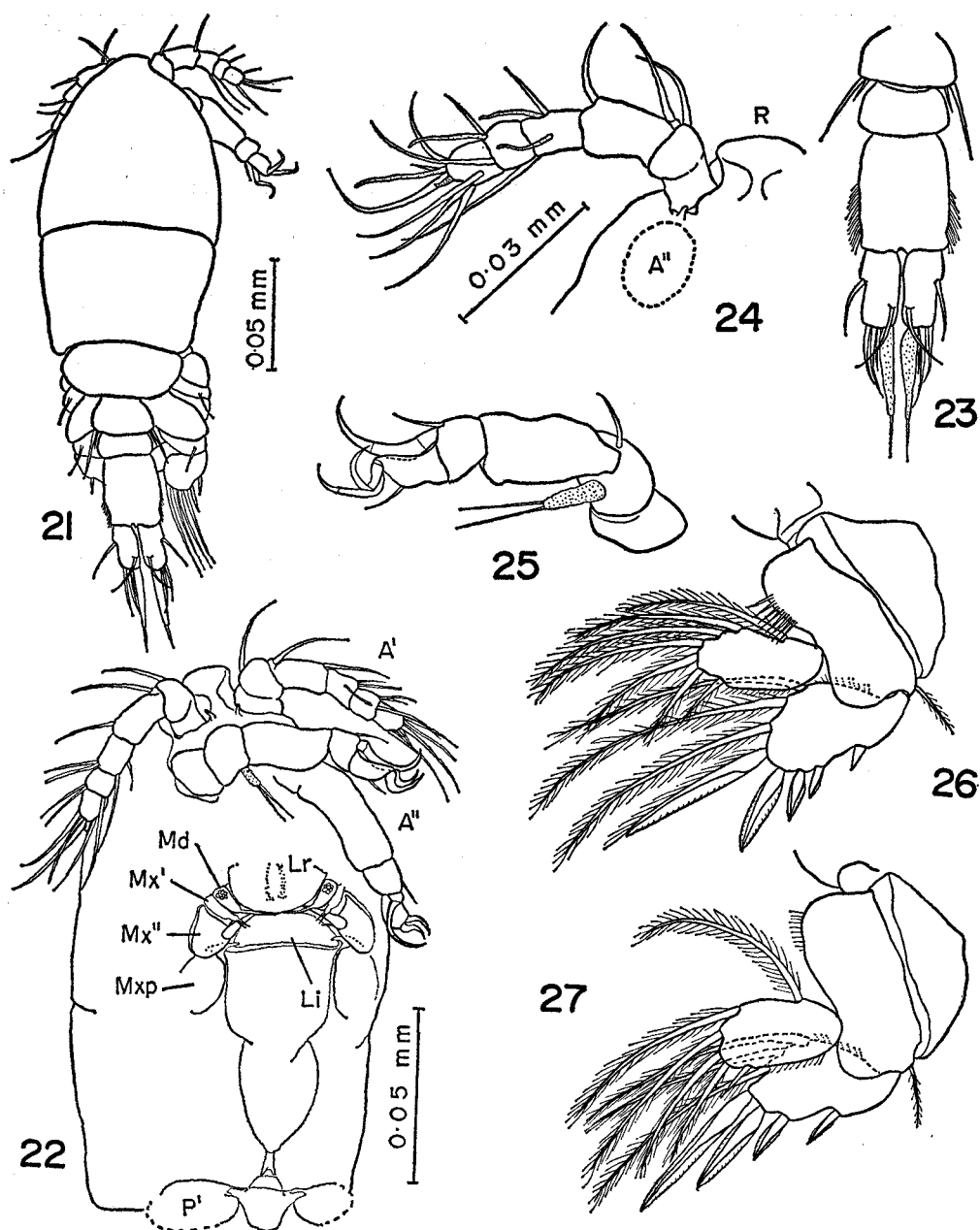
Body length (excluding caudal rami) 0.21 mm, carapace length 0.12 mm and width 0.08 mm on an average of 17 measurements.

Body composed of elongated cephalosome bearing appendages from first antenna through first leg, metasome of second pedigerous segment, and narrow urosome of two segments and anal somite. Cephalosome one and a half as long as wide, longer than metasome plus urosome, and divided distinctly into two parts by a medial transverse suture on the dorsal surface. First urosomal segment bears rudimentary third legs each represented by two setae. Anal somite elongated, with a row of hairs in the posterior half on each side; caudal rami distinct.

First antenna (Fig. 24) uniramous, 5-segmented, with about a dozen setae and five setiform aesthetes.

Second antenna (Fig. 25) biramous. Protopodite 2-segmented, with a seta at the antero-distal corner of basipodite. Endopodite 3-segmented; first segment unarmed and longer than combined length of two distal segments, second segment with a claw and a seta at the antero-distal corner, terminal segment narrower than proximal two, with two terminal claws and a seta near the outer distal edge.

Mouth parts (Fig. 22) weakly developed. Labrum semicircular and rounded



Figs. 21–27. First copepodid stage of *Colobomatus pupa* Izawa. 21. Dorsal view of the body. 22. Ventral view of cephalothorax. 23. Dorsal view of urosome, magnification as in Fig. 22. 24. First antenna, ventral view. 25. Second antenna, ventral view; magnification as in Fig. 24. 26. First leg, ventral view; magnification as in Fig. 24. 27. Second leg, ventral view; magnification as in Fig. 24. Abbreviations: A' = first antenna, A'' = second antenna, Li = labium, Lr = labrum, Md = mandible, Mx' = first maxilla, Mx'' = second maxilla, Mxp = maxilliped, P' = first leg, R = rostrum.

posteriorly. Mandible located just lateral to labrum, 1-segmented and consisting of small, but somewhat elongated basal portion and a thin setiform process extending medially. A small tissue mass, probably a rudiment of exopodite, attached to the base of this appendage. First maxilla feeble, almost as long as the basal portion of mandible, and ending in two fine setules. Second maxilla 2-segmented; basal segment triangular, broad at the base and tapered distally, second segment in a setiform process extending antero-medially and with a fine setule on the inner side near the base. A swelling just behind each second maxilla is probably rudimentary maxilliped and seems to be furnished with an extremely feeble, setiform, lamella at the anterior end.

Two pairs of legs (Figs. 26 & 27) biramous and almost the same in size and structure, each consisting of 2-segmented protopodite and 1-segmented rami. In first leg, basipodite with a plumose seta on the outer margin and a row of simple setae on the distal margin near the inner corner. Exopodite with a row of hairs on the inner margin in addition to eight setae; of these proximal one on the outer margin is simple, three ones distal to it are spatulate, the central seta is spatulate on the outer side but hairy on the inner side, and three on the inner margin are plumosed. Endopodite with seven plumose setae and a row of hairs on the outer margin. In second leg, basipodite with a plumose seta on the outer margin and a row of hairs at the inner distal corner. Exopodite with a row of hairs on the inner margin and seven setae; of these four on the outer margin are spatulate while three on the inner margin are plumose. Endopodite with a row of hairs on the outer margin in addition to five plumose and one simple setae.

Caudal ramus carries six setae, of which the inner-most one on the distal margin is lamellate and tipped with a fine blade.

Discussion

The free-living larvae of *Colobomatus* described in this paper, consisting of five nauplius and the first copepodid stages, bear the features of cyclopoid larvae and closely resemble those of *Sarcotaces*. The first copepodid of *C. pupa* is, however, provided with mouth-parts which is lacking completely in the corresponding stage of *Sarcotaces*. Though they are more or less feeble, the mouth-parts of *Colobomatus* are composed of a full set of oral appendages: mandible, two maxillae and maxilliped in addition to labrum and labium. This structure, characteristic of poecilosstome mouth-parts, resembles that of the Chondracanthidae (Heegaard, 1947).

The larval morphology of *Colobomatus* mentioned above and that of *Sarcotaces* shown in the previous paper (Izawa, 1973) seem to show distinctly a close affinity between these two genera. Further, an affinity between this family and Chondracanthidae is suggested.

Comparing the oral appendages of the first copepodid to those of adult, it is recognized at once that a rather large change takes place differently in sexes in further development. In the female, oral appendages are surrounded by an oral tube

composed of the labrum and an extension of the body wall, and the maxilla, probably the second, has developed into the most powerful appendage. On the other hand, in the male, oral appendages are devoid of any surrounding structure, and the mandible has greatly developed into a conspicuous appendage bearing a stout claw. Noble, Collard and Wilkes (1969) who described *C. embiotocae* and referred to the appendages of the adult female, regarded the second maxilla of the present author as the first maxilla. However, it is difficult to expect that the first maxilla develops into a so powerful appendage. Although the mouth-parts of adult *Sarcotaces* resemble those of *Colobomatus*, any apparent mouth-parts are not yet definable in the first copepodid of *Sarcotaces*, but only a rudiment of mandible. The oral appendages must appear in *Sarcotaces* in later stages; it is, however, interesting to note that the mandible once degenerated develops again into a strong appendage in adult males.

The eggs of *Colobomatus* are more or less smaller than those of *Sarcotaces*, and therefore both the nauplii and the first copepodid of the former are smaller than those of the latter. The number of eggs produced by a single female is, however, much different between the two genera, a few hundreds at the maximum in *Colobomatus*, but over ten thousands in *Sarcotaces*. Such a difference seems to accord chiefly with the body size of adult females and further with the space where they live.

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Summary

1. The free-living larval stages of *Colobomatus pupa* are described and figured on the larvae reared under the laboratory conditions.

2. The larvae passed to the first copepodid through five nauplius stages within 5 days at 16–20°C. Their features are characteristic of cyclopoid larvae and closely resemble those of *Sarcotaces*.

3. The nauplii have three pairs of appendages which are, however, simplified and devoid of feeding apparatus, gnathobase and masticatory setae. No rudiments of post-mandibular oral appendages are recognized. The rudimentary first and second legs appear at the fifth stage. Larvae are non-feeding throughout all stages. No growth of the body is gained, rather the body width diminishes slightly in last two stages.

4. The first copepodid swims actively, probably this may be the infective stage. Though they are feeble, the mouth-parts are present and consist of mandibles, two

maxillae and rudimentary maxillipeds. This structure is characteristic of poecilostome mouth-parts and actually resembles that of the chondracanthid first copepodid. The first pedigerous segment is separated, on the dorsal surface, from the anterior half of cephalothorax by a distinct suture.

5. A close affinity between *Colobomatus* and *Sarcotaces* is distinct, and thus Delamare-Deboutteville's classification (1962) to include the gall-forming genera, *Sarcotaces* and *Ichthyotaces*, in the family Philichthyidae is confirmed. This family is referred to poecilostome Cyclopoida and related to the family Chondracanthidae.

6. In adults, the second maxilla in the female and mandible in the male are the most powerful and functional oral appendages.

7. Eggs of *Colobomatus* are generally slightly smaller than those of *Sarcotaces* and then the free-living larvae are smaller in the former than in the latter. Number of eggs is much less in the former than in the latter, this probably accord with the body size of adult females and further with space where they live.

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